g) and water (20 ml) in a rapid current of steam. The distillate was extracted with ether and the extract distilled; I (X = Br) was collected at 45–47° (5 mm), $n^{25}D$ 1.5293 (3.45 g, 53%).

Anal. Calcd for C_8H_8BrF: C, 47.32; H, 3.97; Br, 39.35; F, 9.36. Found: C, 47.48; H, 4.05; Br, 39.28; F, 9.43.

3,5-Dimethyl-4-fluoroiodobenzene (I; X = I). Nitrosylsulfuric acid, prepared from concentrated sulfuric acid (12 ml) and sodium nitrite (30 g), was added to I (X = NH_2) (4.2 g) in concentrated sulfuric acid (15 ml) at 20°, and the mixture was poured into a solution of potassium iodide (20 g) in water (40 ml). After 30 min at 50°, I (X = I) was isolated with ether and distilled, bp 90° (0.5 mm).

Anal. Calcd for C_8H_8FI: C, 38.43; H, 3.22; F, 7.60; mol wt, 250. Found: C, 38.50; H, 3.11; F, 7.33; mol wt (mass spectroscopy), 250.

3,5-Dimethyl-4-fluorobenzonitrile (I; X = CN). Sodium nitrite (14 g) was added to a cold solution of I (X = NH_2) (22.8 g) in concentrated hydrochloric acid (40 ml) and water (40 ml). The solution was neutralized with sodium carbonate and then added dropwise to cuprous cyanide (18 g) dissolved in water (100 ml) containing potassium cyanide (18 g). The resulting mixture was warmed to 50° for 2 hr and extracted with ether; the extract was steam distilled. Ether extraction gave I (X = CN) (12.9 g, 55%), mp 97–97.5° after crystallization from methanol.

Anal. Calcd for C_9H_8FN: C, 72.47; H, 5.41; F, 12.74; N, 9.39. Found: C, 72.67; H, 5.41; F, 12.52; N, 9.20.

2,6-Dimethyl-4-fluorobenzoic Acid (I; X = COOH). A solution of I (X = CN) (4.5 g) in concentrated sulfuric acid (12.5 ml) was heated 5 hr at 80°; water (50 ml) was then added and the crystalline amide (4.0 g) collected, mp 140–147°. The amide (3.8 g) was heated 30 min at 150° with phosphoric acid (10 ml), then cooled, made alkaline with potassium hydroxide solution, filtered, and acidified. I (X = COOH) (2.25 g, 50%) was crystallized from benzene, mp 146.5–148°.

Methyl 2,6-Dimethyl-4-fluorobenzoate (I; X = COOMe). Methylation of I (X = COOH) with diazomethane in ether gave I (X = COOMe), bp 57–58° (4 mm), mp 29–30°.

Anal. Calcd for $C_{10}H_{11}FO_2$: C, 65.92; H, 6.09; F, 10.43; mol wt, 182. Found: C, 65.70; H, 5.96; F, 10.40; mol wt (mass spectroscopy), 182.

2,6-Dimethyl-4-fluorophenol (I; X = OH). Sodium nitrite (3.5 g) was added to a solution of I (X = NH_2) (5.6 g) in 3 *N* sulfuric acid (40 ml), and the resulting solution was filtered and added dropwise to boiling 70% sulfuric acid (200 ml) in a current of steam. Ether extraction of the distillate, followed by sublimation at 46–47° (0.1 mm), gave I (X = OH) (0.96 g), mp 72–76°, raised by recrystallization from Skellysolve B to 81–82°.

Anal. Calcd for C_8H_9FO: mol wt, 140. Found: mol wt (mass spectroscopy), 140.

Nucleosides. XXXVIII. Proton Magnetic Resonance Studies of Acetylated Nucleosides[1]

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Contribution from the Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York, New York. *Received August 12, 1966*

Abstract: The proton magnetic resonance (nmr) spectra of 36 acetylated derivatives of 3′-aminohexosyl and pentosyl nucleosides were examined. The results show that chemical shifts of acetyl signals are unreliable for determining configuration of the sugar moiety. The effect of conformation and neighboring anisotropy on the acetyl chemical shifts was studied. Removal of the anisotropy of the 5,6 double bond of the aglycon by hydrogenation produced an effect on specific signals which, together with evidence from partially acetylated compounds, enabled individual resonances to be assigned. A general rule based on the effect of the anisotropy of the 5,6 double bond on the C_2′-acetoxy resonance is proposed which may have wide application in the assignment of anomeric configuration to pyrimidine nucleosides. Upon hydrogenation of the 5,6 double bond, a diamagnetic (upfield) shift of the $C_{2'}$-acetoxy resonance signal is observed in pyranosyl pyrimidine nucleosides having *cis*-$C_{1'}$–$C_{2'}$ substituents and pentofuranosyl pyrimidine nucleosides having a *trans*-$C_{1'}$–$C_{2'}$ relationship. Removal of the 5,6 double bond in pyranosyl pyrimidine nucleosides having a *trans*-$C_{1'}$–$C_{2'}$ relationship and pentofuranosyl pyrimidine nucleosides having a *cis*-$C_{1'}$–$C_{2'}$ relationship causes a paramagnetic (downfield) shift in the $C_{2'}$-acetoxy resonance signal. Several new acetylated nucleoside derivatives were prepared.

Previous reports from this laboratory[2–5] dealt with the synthesis and structure proof of several 3′-amino-3′-deoxy-β-D-*aldo*-hexopyranosyl nucleosides of purines and pyrimidines. Because of inconsistencies in the acetyl resonance signals in the nmr spectra of several of these nucleoside derivatives, we resorted to *chemical* studies to determine the configuration of the glycosyl moieties.

In 1958, Lemieux and co-workers[6] studied the nmr spectra of a number of acetylated pyranoses and inositols of known configuration and found that, as a rule, axial acetoxy groups absorb at lower field than equatorial acetoxy groups. These studies have been largely confirmed by other investigators.[7–10]

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748). Preliminary reports have appeared: R. J. Cushley, K. A. Watanabe, and J. J. Fox, *Chem. Commun.*, 598 (1966); Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, p 39D.

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Lichtenthaler[11-13] compiled data on the chemical shifts of acetyl methyl resonances of a large number of aminocyclitols. More recently, Lichtenthaler, *et al.*,[14-16] used acetyl methyl resonances for the assignment of configuration to acetylated derivatives of 3′-amino-3′-deoxy-β-D-*aldo*-hexopyranosyl nucleosides. The chemical shift ranges they found are given in Table I.

Table I. Signal Ranges of Polyacetates of Amino Sugars and 3′-Amino Sugar Nucleosides as Proposed by Lichtenthaler, *et al.*[13,16]

Group	Acetyl resonance chemical shift, τ DMSO-d_6	$CDCl_3$[c]
Axial acetoxy	7.86–7.91[a]	7.78–7.87
Equatorial acetoxy	7.95–8.11[a]	7.94–8.02
Axial acetamido	7.96–8.14[b]	7.92–7.94
Equatorial acetamido	8.19–8.30[a]	8.05–8.09

[a] Data from ref 16a. [b] Data compiled from ref 16b. [c] Data from ref 13.

From our data (Table II) it is apparent that the assignment of configuration from signal ranges of acetoxy substituents in 3′-amino sugar nucleosides is hazardous. For example, 1-(3′-acetamido-tri-O-acetyl-3′-deoxy-β-D-galactopyranosyl)uracil (XI, Table II) possesses two acetyl signals at τ 8.03, one at 8.10, and one at 8.23. If these values are applied to the ranges in Table I, one would conclude that XI contains no axial acetoxy functions when, in fact, this nucleoside isomer contains an axial acetoxy group at $C_{4'}$. The 1-(tri-O-acetyl-3′-amino-3′-deoxy-β-D-glucopyranosyl)uracil hydrochloride (VI),[4] which contains only equatorial acetoxy groups, gives acetyl resonances at τ 7.85, 7.98, and 7.98. Yet, ranges listed in Table I would have warranted the assignment of the downfield acetoxy resonance to the axial configuration. Moreover, the 5,6-dihydro derivative (X) of 1-(3′-acetamido-2′-O-acetyl-3′-deoxy-mannopyranosyl)uracil shows acetyl signals at τ 8.13 and 8.22 which, from the ranges in Table I, would have implied only equatorial acetyl groups. Yet, compound X, in fact, contains one axial acetoxy group. Similarly the 5,6-dihydro derivative of the manno nucleoside tetraacetate (VIII) has all its acetyl signals in Lichtenthaler's "equatorial region" (Table I). However, it is known that the 2′-acetoxy group has the axial orientation.

In a previous paper[3] we reported acetyl resonances for several tetraacetates of 9-(3′-amino-3′-deoxy-β-D-*aldo*-hexopyranosyl)adenines of known configuration. Several of these signals fall outside the ranges listed in Table I. To accommodate these signals it would be necessary to further broaden the ranges to regions of overlap which would serve to increase the ambiguities in the configurational assignments. In fact, on the basis of nmr acetyl signals, Lichtenthaler, *et al.*,[14] had assigned the *talo* configuration to 9-(3′-acetamidotri-O-acetyl-3′-deoxy-β-D-*aldo*-hexopyranosyl)hypoxanthine which they [16a] later revised to the *gluco* configuration.

Recent studies by Lemieux and Stevens[17] also point to exceptions to the rule that axial acetoxy groups occur to lower field than equatorial acetoxy groups. It is clear that, without other compelling evidence, acetyl resonance signals cannot be relied upon to establish configuration without ambiguity in the carbohydrate area in general and in the nucleoside area in particular. It is important to note that with regard to chemical shifts not only is the configuration of the acetyl group(s) in question important but also its electronic environment. The purpose of this report is to examine some of these factors which may affect acetyl resonance signals in the nucleoside area.

The main factors which were considered to affect the acetyl resonance signals of these nucleoside derivatives were: conformation of the carbohydrate moiety, anisotropic effect of groups other than the aglycon, and anisotropic effect of the aglycon.

To study the effect of conformation on the acetyl resonance signals, 4′,6′-benzylidene derivatives of 1-(3′-acetamido-2′-O-acetyl-3′-deoxy-β-D-glucopyranosyl)-uracil (XIII)[5] and 1-(3′-acetamido-2′-O-acetyl-3′-deoxy-β-D-mannopyranosyl)uracil (XIV), where the carbohydrate moiety would be a perfect chair form, were compared with their respective 2′,3′-diacetates (III,[5] IX) and 2′,3′,4′,6′-tetraacetates (I,[5] VII). The $C_{2'}$-acetoxy resonance in the two series, *gluco* and *manno*, should reflect only differences, if any, due to deformation of the chair conformation. For the *gluco* compounds, I, III, and XIII, the $C_{2'}$-acetoxy resonance signals were τ 8.11, 8.10, and 8.10, respectively, while for the *manno* derivatives, VII, IX, and XIV, the $C_{2'}$-acetoxy resonance signals were τ 7.92, 7.90, and 7.93, respectively. From this data we conclude that the effect of deformation (if any) of the sugar moiety on chemical shifts is negligible. This result is not surprising since, in all instances, the groups are either all equatorial or only one of them is axial.

The second factor, anisotropic effect of groups other than the aglycon, is seen most strikingly by comparing 1-(3′-acetamido-tri-O-acetyl-3′-deoxy-β-D-glucopyranosyl)uracil (I) with 1-(tri-O-acetyl-3′-amino-3′-deoxy-β-D-glucopyranosyl)uracil hydrochloride (VI). Replacement of an acetamido group by an ammonium group caused the $C_{2'}$-acetoxy signal at τ 8.11 and the $C_{4'}$-acetoxy signal at τ 7.99 to be shifted downfield by 0.13 and 0.14 ppm, respectively. Had one not known the configuration of VI, one could have assigned the axial configuration to the 4′-acetoxy function on the basis of chemical shift ranges listed in Table I. A similar downfield shift has been observed[18] when the acetamido groups in fully acetylated deoxystreptamine were replaced by ammonium functions.

On the other hand, from an examination of the compounds listed in Table II, it is seen that there is an effect on the N-acetyl resonance signal owing to different vicinal substituents. In general, one neighboring acetyl

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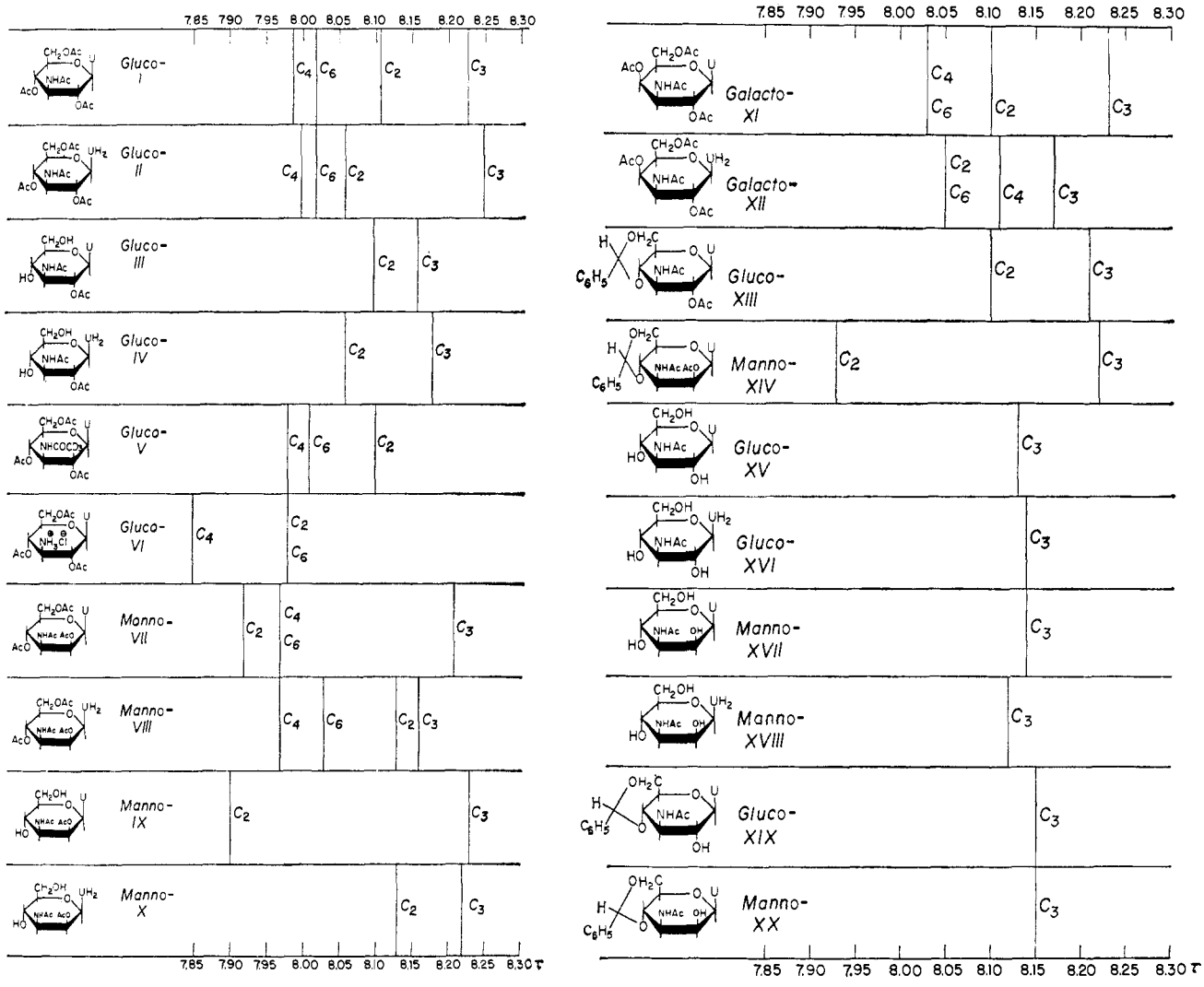
Table II, Acetyl Resonances of Acetylated Derivatives of 1-(3′-Amino-β-D-*aldo*-hexopyranosyl)uracils in DMSO-d_6[a]



[a] C subscript refers to the position on the sugar moiety. U, uracil; UH_2, 5,6-dihydrouracil.

group causes a diamagnetic shift in the acetyl signal of up to 0.1 ppm. Anet and co-workers[10] have reported that acetylation of a hydroxyl group causes a shift in the resonance of a vicinal N-acetyl of +0.06 ppm. A second vicinal acetoxy group does not appreciably increase the diamagnetic effect. These effects are seen, for example, when compound XVII is compared with XX,[5] IX, and VII, and compound XV is compared with XIX,[5] III, and I.

Finally, to study the anisotropic effect of the aglycon on the chemical shifts of acetyls, we prepared the 5,6-dihydro derivatives of 15 nucleosides. Removal of anisotropy has often been useful[19] in the assignment of individual resonances. This anisotropic effect is contingent upon restricted rotation about the glycosyl bond. Such restriction of rotation has been proposed recently.[20,21] An examination of molecular models shows this restricted rotation to be applicable in pyranosyl as well as in furanosyl nucleosides. (It must be emphasized that pure conformers are not required but simply a substantial population in a preferred conformation). The equation of McConnell[22] shows that the anisotropy effect is maximal at the symmetry axes and decreases by the reciprocal of the third power of the distance between the axis and the group in question.

A comparison of the nmr data of the nucleosides, their dihydro analogs, and their partially acetylated derivatives (Table II) permits definitive assignment of peaks to individual acetyl resonances. A schematic representation of the anisotropic effect of the aglycon is depicted in Figure 1. In structure A (the *gluco* isomer, I) the $C_{2'}$-acetoxy group is in an area of positive shielding. The $C_{3'}$-, $C_{4'}$-, and $C_{6'}$-acetyl groups are in an area of negative shielding. Therefore, removal of the anisotropic effect of the aglycon by hydrogenation of the 5,6 double bond should result in a deshielding effect (downfield chemical shift) of the 2′-acetoxy substituent and a shielding effect on the remaining acetyl groups. With the *manno* derivative (Figure 1, structure B), the 2′-acetoxy substituent is in an area of high negative shielding. In the 5,6-dihydro derivative of B the negative shielding is lost and the $C_{2'}$-acetoxy resonance should shift to higher field.

These considerations are generally borne out by the data in Table II. A comparison of the compound

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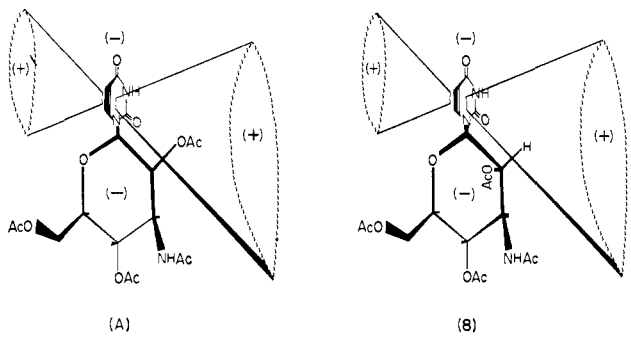
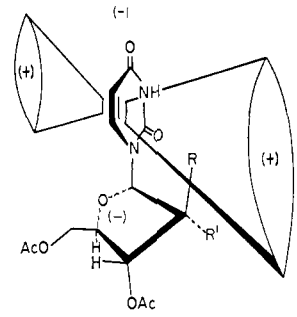


Figure 1.



XXV, *arabino*, R=OAc, R'=H
XXIII, *ribo*, R=H, R'=OAc

Figure 2.

pairs I–II[5] and III–IV (type A compounds) shows that removal of anisotropy caused a downfield shift of the $C_{2'}$-acetoxy substituent by 0.05 ppm while the N-acetyl resonance shifted to higher field. A similar upfield shift of the 3'-acetyl is produced when the monoacetylated *gluco* nucleoside XV[4] is compared to its dihydro derivative XVI.[4]

In the case of *manno* nucleosides (type B compounds) the effect is even more striking. A comparison of the compound pairs VII–VIII and IX–X shows that the loss of anisotropy produced upfield shifts of 0.21 and 0.23 ppm for the $C_{2'}$ axial acetoxy group in VIII and X, respectively. It should be noted that the chemical shifts for the $C_{2'}$-acetoxy groups in VIII and X fall in the region designated[16] in Table I for equatorial acetates. The $C_{3'}$-acetyl group of VII is shifted downfield by 0.05 ppm in its dihydro analog VIII. This unexpected paramagnetic shift is found in four pairs of nucleoside–dihydronucleosides (VII–VIII, IX–X, XI–XII, and XVII–XVIII), all of which contan an axial C–O bond vicinal to the 3'-N-acetyl group. Apparently, axial substituents in proximity to regions of maximum negative shielding exert a diamagnetic effect on neighboring equatorial groups. Thus, in the *galacto* series in going from XI to XII the $C_{3'}$-acetyl signal at highest field is shifted downfield by 0.06 ppm in accordance with the preceding observations in the *manno* series,

The $C_{2'}$-acetoxy group was assigned to the τ 8.10 signal by comparison with compound I. The 6-proton signal at τ 8.03 was assigned to the $C_{4'}$- and $C_{6'}$-acetoxy groups. As expected from the discussion of Figure 1, structure A (*vide supra*), the $C_{2'}$-acetoxy signal is shifted downfield by 0.05 ppm in going from XI to XII. Similarly the axial $C_{4'}$-acetoxy signal is shifted upfield from τ 8.03 to 8.11, as expected. The 6-proton signal at τ 8.05 in XII can now be assigned to the $C_{2'}$- and $C_{6'}$-acetoxy groups. It should be noted that the axial $C_{4'}$-acetoxy group is in the "so-called" equatorial range[16] for both XI and XII even though it is in an area of deshielding in compound XI. In compound XII (also in compound VIII) the signal for the equatorial $C_{3'}$-acetamido group occurs in the proposed[16] axial range. Compounds XI and XII can be compared with 1-(tetra-O-acetyl-β-D-galactopyranosyl)uracil (τ 7.80, 7.98, and 8.02 (6 H)) and 1-(tetra-O-acetyl-β-D-galactopyranosyl)-5,6-dihydrouracil (τ 7.85, 8.00 (6 H), and 8.05). The latter two compounds show three signals each in the equatorial acetoxy resonance region (Table I) and one each, τ 7.80 and 7.85, respectively, slightly below the axial acetoxy resonance region in Table I assigned to the $C_{4'}$-acetoxy group. The difference of 0.05 ppm in the $C_{4'}$-acetoxy signal in galactosyluracil tetraacetate *vs.* its dihydro derivative is consistent with the upfield shift one would predict from the anisotropic effect as portrayed in Figure 1.

Lichtenthaler has assigned the N-acetyl resonance (Table I) to the highest field signals purely on an empirical basis. Horton[23] assigned the highest field signal to the primary 6-acetoxy group in the α and β anomers of 2-acetamido-2-deoxy-D-glucopyranose tetraacetates, Our study on 1-(3'-acetamido-d_3-tri-O-acetyl-3'-deoxy-β-D-glucopyranosyl)uracil (V) supports Lichtenthaler's assignment (compare V with I) and confirms the assignment of the resonance signal at τ 8.23 in I to the N-acetyl group.

Recent studies by Hall[24] on acetylated methyl pentofuranosides showed that the chemical shifts of the acetyl resonances furnish no indication of configuration, However, in our studies of acetylated derivatives of 1-β-D-aldopentofuranosyluracils and -thymines (cytosine compounds can be studied by deamination to their respective uracil derivatives), information regarding configuration at the anomeric center was readily obtained. With the pentofuranosyl nucleosides a conformational situation similar to that of the pyranosyl nucleosides obtains. Thus, the compounds exist in a preferred conformation in which the 5,6 double bond of the aglycon is "*endo*" to the five-membered sugar ring[20,21] as shown in Figure 2 for the *ribo* (XXIII) and *arabino* (XXV) derivatives. It will be noted that now the *cis*-acetoxy group (*arabino*, R = OAc, R' = H) is found to be in the cone of positive shielding of the 5,6 double bond. An examination (Table III) of two C_1–$C_{2'}$-*cis* nucleoside–dihydronucleoside pairs (*arabino* XXV and XXVI and *lyxo* XXVII and XXVIII) shows that the $C_{2'}$-acetoxy resonance is shifted downfield by 0.05 and 0.10 ppm, respectively, upon hydrogenation. Assignment of the $C_{2'}$ resonance signals to highest field in XXV and XXVII also derives from the absence of such signals in the 2'-deoxynucleosides XXI and XXII. As previously noted with the pyranosylnucleosides, the $C_{3'}$- and $C_{5'}$-acetoxy resonances in XXI are not appreciably shifted when anisotropy of the 5,6 double bond is removed. In the *ribo*- and *xylo*-nucleoside pairs (XXIII and XXIV, XXIX and XXX, and XXXI and XXXII) where a *trans* relationship exists between the C_1–$C_{2'}$ substituents, either an upfield shift is observed in the $C_{2'}$-

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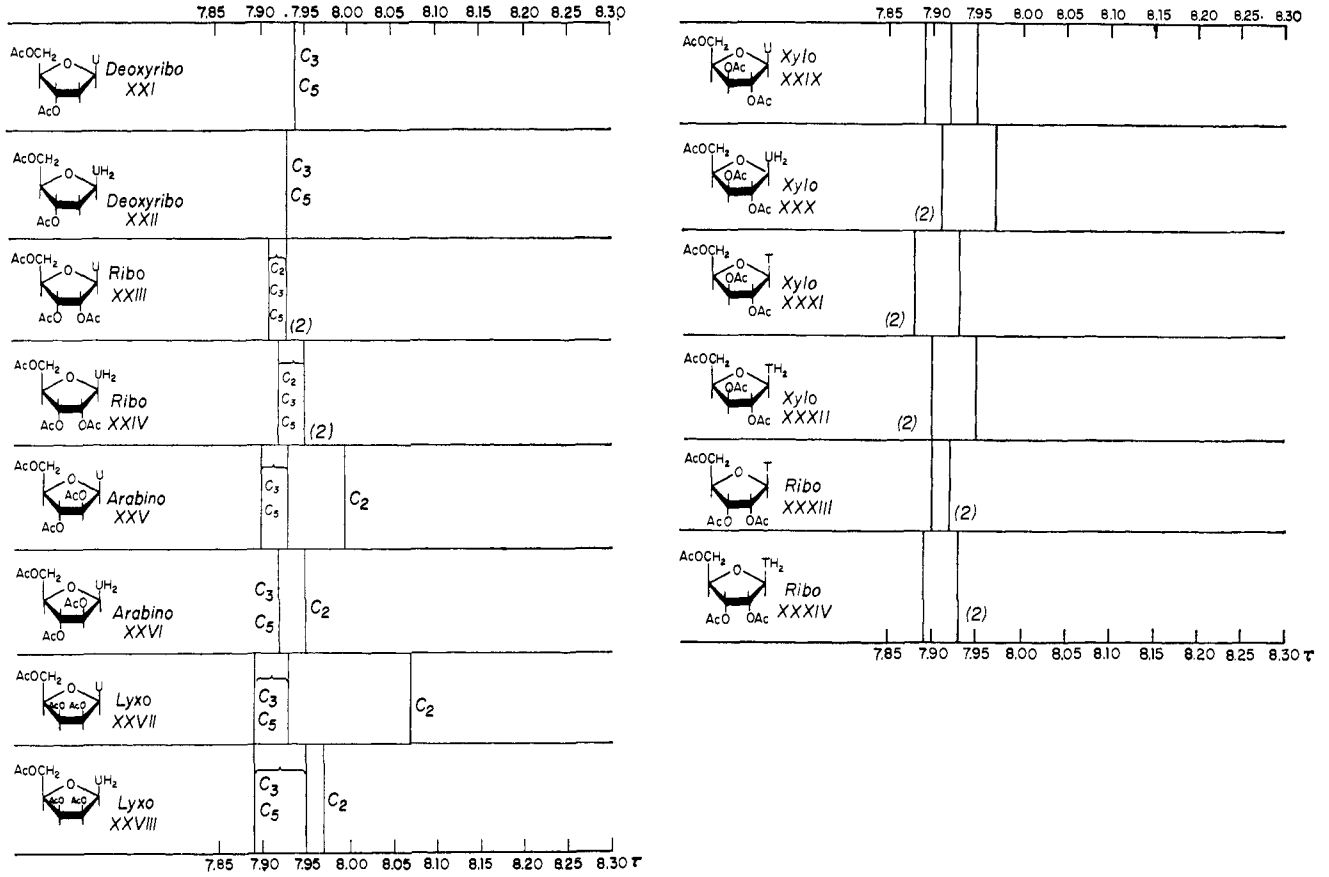
(24) L. D. Hall, *Advan. Carbohydrate Chem.*, **19**, 67 (1964).

Table III. Acetyl Resonances of Acetylated Derivatives of 1-(β-D-*aldo*-Pentofuranosyl)nucleosides in DMSO-d_6[a]



[a] C subscript refers to the position on the sugar moiety. Numbers in parentheses refer to number of acetyl signals at a particular τ value. U, uracil; UH_2, 5,6-dihydrouracil; T, thymine; TH_2, 5,6-dihydrothymine.

acetoxy resonance or (XXXIII and XXXIV) no appreciable effect within experimental error is seen.

An important generalization emerges from these studies which has application in the nucleoside area. With the pyranosyl pyrimidine nucleosides, when the $C_{2'}$-acetoxy group and the pyrimidine are in a *cis* relationship, the $C_{2'}$-acetoxy resonance will be shifted upfield by 0.21–0.23 ppm when the 5,6 double bond is hydrogenated. When a *trans*-diequatorial relationship obtains at $C_{1'}$–$C_{2'}$, there is a small but significant downfield shift of the $C_{2'}$-acetoxy signal upon removal of unsaturation.

With acetylated pentofuranosyl nucleosides, a *cis*-$C_{1'}$–$C_{2'}$ relationship causes a paramagnetic shift in the $C_{2'}$-acetoxy resonance upon saturation of the double bond, whereas by similar treatment the *trans* nucleosides will exhibit a small diamagnetic shift.

Lemieux and Lineback[25] stated that anomeric configuration of furanosides cannot be determined from coupling constants for the C_1–C_2 protons. From recent studies, Nishimura and Shimizu[26] proposed that anomeric assignment in nucleosides can be made by comparing the chemical shifts of the anomeric protons of nucleoside anomers. They found that the C_1 proton occurs to lower field when the 1′–2′ substituents are *cis* than when they are *trans*. Unfortunately, application of their method requires *both* the α and β anomers in every case.

Our method should have wide application for the assignment of anomeric configuration to pyrimidine nucleosides. Only one anomer is required for such study which, by acetylation and hydrogenation, is converted to the necessary derivatives for the determination of its anomeric configuration.

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Experimental Section

General Procedures. Proton magnetic resonance (nmr) spectra were taken on a Varian A-60 spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal reference. Values are given in τ and are accurate to ±0.01 ppm. All thin layer chromatography (tlc) was effected with Eastman chromagram sheets, Type K 301R (silica gel) with the systems: A, butanol–acetic acid–water (5:2:3); B, butanol–water (86:14); and C, methanol–benzene (1:9). Spots were visualized with ultraviolet light. Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Ultraviolet spectra were determined in ethanol on a Cary 15 spectrophotometer. Infrared spectra were determined on an Infracord spectrophotometer in a KBr pellet. Elemental analyses were made by Spang Microanalytical Laboratory, Ann Arbor, Mich.

1-(3′-Acetamido-d_3-3′-deoxy-β-D-glucopyranosyl)uracil. To 4 ml of water and 0.5 ml of methanol was added 220 mg (0.8 mmole) of 1-(3′-amino-3′-deoxy-β-D-glucopyranosyl)uracil.[4] Hexadeuterioacetic anhydride (0.1 ml) was added, and the mixture was shaken. After 5 min solution was complete. The solution was kept at 0° in a refrigerator for 2 hr and then evaporated to dryness *in vacuo*. The resulting ninhydrin-negative solid was crystallized from 1 ml of methanol to yield 150 mg (59%) of 1-(3′-acetamido-d_3-3′-deoxy-β-D-glucopyranosyl)uracil as colorless needles, mp 177–179°.

Anal. Calcd for $C_{12}H_{14}(D_3)N_3O_7 \cdot H_2O$: N, 12.49. Found: N, 12.48.

1-(3′-Acetamido-d_3-tri-O-acetyl-3′-deoxy-β-D-glucopyranosyl)uracil (V), The N-deuterioacetate (86 mg, 0.27 mmole) was added to 0.4 ml of pyridine and treated with 0.07 ml of acetic anhydride. The mixture was heated at 80° for 25 min until solution was complete. After standing overnight at room temperature the solution was evaporated to dryness *in vacuo*. The product was crystallized from acetone to yield 100 mg (83%) of V, mp 250–251°. Tlc in solvent system A showed one spot, R_f 0.28.

Anal. Calcd for $C_{18}H_{20}(D_3)N_3O_{10}$: C, 48.65; H, 5.90; N, 9.45. Found: C, 48.22; H, 5.30; N, 9.36.

1-(3′-Acetamido-3′-deoxy-β-D-mannopyranosyl)uracil (XVII), A solution of 1-(3′-acetamido-4′,6′-O-benzylidene-3′-deoxy-β-D-mannosyl)uracil (XX) (0.830 g, 2.06 mmoles) in methanol (49 ml) and acetic acid (1 ml) was shaken with 10% palladium–charcoal (0.91 g) overnight in a hydrogen atmosphere. The catalyst was removed by filtration. The filtrate was evaporated to a syrup, which crystallized from ethanol as colorless needles (0.47 g, 72%), mp 185–187° dec.

Anal. Calcd for $C_{12}H_{17}N_3O_7$: C, 45.71; H, 5.39; N, 13.33. Found: C, 45.73; H, 5.42; N, 13.32.

This compound (XVII) was previously reported as a syrup.[5] This debenzylidenation procedure was not applicable for the *gluco* derivative XIX which was insoluble in methanol–acetic acid.

1-(3′-Acetamidotri-O-acetyl-3′-deoxy-β-D-mannopyranosyl)uracil (VII), Acetylation of compound XVII (200 mg, 0.63 mmole) was carried out in a mixture of pyridine (20 ml) and acetic anhydride (10 ml) overnight at room temperature. The solvent was evaporated to a syrup which was dissolved in 20 ml of benzene and again evaporated. The residue was crystallized from ethanol–petroleum ether to afford 254 mg of colorless needles (84%), mp 237–239° dec.

Anal. Calcd for $C_{18}H_{23}N_3O_{10}$: C, 48.98; H, 5.25; N, 9.52. Found: C, 48.62; H, 5.39; N, 9.53.

1-(3′-Acetamido-2′-O-acetyl-4′,6′-benzylidene-3′-deoxy-β-D-mannopyranosyl)uracil (XIV). Compound XX[5] (200 mg, 0.5 mmole) was dissolved in 4 ml of pyridine and treated dropwise with acetic anhydride (0.15 ml). The solution was heated at 30–35° for 17 hr after which it was evaporated to dryness *in vacuo* at 70°. The resulting syrup was crystallized from 4 ml of ethanol. After one recrystallization from ethanol, 193 mg (87%) of white needles was obtained, mp 297–298° dec with prior darkening at 290°.

Anal. Calcd for $C_{21}H_{23}N_3O_8$: C, 56.59; H, 5.20; N, 9.43. Found: C, 56.32; H, 5.54; N, 9.28.

1-(3′-Acetamido-2′-O-acetyl-3′-deoxy-β-D-mannopyranosyl)uracil (IX), Compound XIV (107 mg, 0.22 mmole) was dissolved in a mixture of methanol (9 ml) and acetic acid (1 ml) and was dibenzylidenated by hydrogenation with 10% palladium–charcoal (137 mg). After the hydrogen uptake ceased, the catalyst was filtered and washed with 50% aqueous ethanol (10 ml). The combined filtrate and washings were evaporated to a colorless syrup which was crystallized from ethanol, 72 mg (91%), mp 244–249° dec.

Anal. Calcd for $C_{14}H_{19}N_3O_8$: C, 47.06; H, 5.32; N, 11.76. Found: C, 47.25; H, 5.62; N, 11.59.

1-(3′-Acetamidotri-O-acetyl-3′-deoxy-β-D-galactopyranosyl)uracil (XI), 1-(3′-Amino-3′-deoxy-β-D-galactopyranosyl)uracil hydrochloride monohydrate[5] (107 mg, 0.51 mmole) was dissolved in 27 ml of pyridine and was treated with 3 ml of acetic anhydride overnight at room temperature. The reaction mixture was evaporated to dryness under reduced pressure at about 60°. The solvent was removed by three azeotropic distillations with toluene. The residue was crystallized from ethanol, 78 mg (35%), mp 189–192° dec.

Anal. Calcd for $C_{18}H_{23}N_3O_{10}$: C, 48.98; H, 5.25; N, 9.52. Found: C, 49.31; H, 5.53; N, 9.97.

1-(Tri-O-acetyl-β-D-arabinofuranosyl)uracil (XXV), 1-(β-D-Arabinofuranosyl)uracil[27] (0.41 g, 1.68 mmoles) was dissolved at 50° in 3 ml of acetic anhydride and 1 ml of pyridine. The slightly yellow solution was allowed to stand at room temperature for 6.5 hr. Methanol (5 ml) was added and, after 24 hr, the solvents were evaporated *in vacuo*, whereupon crystallization occurred. The solution (~0.5 ml) was placed in a refrigerator for 18 hr. The resulting crystals (445 mg, 66%) were washed with methanol (1 ml) and dried over P_2O_5 (16 hr, 60°) under high vacuum, mp 129–130.5° (lit.[28] mp 129–130°).

(27) J. F. Codington, R. Fecher, and J. J. Fox, *J. Am. Chem. Soc.*, **82**, 2794 (1960).

Anal. Calcd for $C_{15}H_{18}N_2O_9$: C, 48.66; H, 4.90; N, 7.57. Found: C, 48.73; H, 4.90; N, 7.58.

1-(Tri-O-acetyl-β-D-lyxofuranosyl)uracil (XXVII), To a solution of 124 mg (0.51 mmole) of 1-(β-D-lyxofuranosyl)uracil[29] in 4 ml of pyridine was added 0.19 ml of acetic anhydride. After standing 28 hr at room temperature, the solution was evaporated to dryness, and the colorless residue was dissolved in 20 ml of chloroform and washed with 5 ml of water. Evaporation of the solution to dryness gave a colorless syrup (136 mg, 72%). Chromatography (tlc) showed one spot R_f 0.69, system A, and one spot R_f 0.59, system B.

Anal. Calcd for $C_{15}H_{18}N_2O_9$: C, 48.66; H, 4.90; N, 7.57. Found: C, 48.72; H, 5.12; N, 7.51.

1-(Tri-O-acetyl-β-D-xylofuranosyl)uracil (XXXIX), Treatment of 300 mg of 1-(3′,5′-O-isopropylidene-β-D-xylofuranosyl)uracil[30] with 9.6 ml of 0.1 *N* hydrochloric acid in 20 ml of ethanol at 78° for 4 hr yielded a product whose tlc gave one spot R_f 0.59 (starting material R_f 0.66), solvent system A; R_f 0.49 (starting material R_f 0.66), solvent system B. The crude solid, which showed the absence of $C(CH_3)_2$ in the nmr spectrum was crystallized from ethanol to yield 123 mg (48%) of 1-(β-D-xylofuranosyl)uracil, mp 155–159° (lit.[27] mp 158–158.5°).

The 1-(β-D-xylofuranosyl)uracil (123 mg, 0.5 mmole) was dissolved in 4 ml of anhydrous pyridine and 0.25 ml of acetic anhydride. The product, after standing 17 hr at room temperature, was evaporated to dryness, and the colorless syrup was crystallized from ethanol to which petroleum ether (bp 30–60°) was added to turbidity. The resulting amorphous solid (137 mg, 74%) showed one spot (tlc) R_f 0.67, system A, and one spot R_f 0.58, system B.

Anal. Calcd for $C_{15}H_{18}N_2O_9$: C, 48.66; H, 4.90; N, 7.57. Found: C, 48.34; H, 5.18; N, 7.57.

1-(Tri-O-acetyl-β-D-xylofuranosyl)thymine (XXXI), To 5 ml of pyridine was added 200 mg (0.78 mmole) of 1-(β-D-xylofuranosyl)-thymine[31] and 0.25 ml of acetic anhydride. The solution was refluxed 30 min and allowed to stand at room temperature for 18 hr. The solution was evaporated to dryness, dissolved in 5 ml of chloroform, and washed twice with 1 ml of saturated sodium chloride solution, and once with 1 ml of water. The chloroform layer was evaporated to dryness to yield a glass (236 mg, 79%). Tlc showed one spot R_f 0.70, system A, and one spot R_f 0.60, system B. Nmr studies showed the presence of 0.5 mole of water of crystallization.

Anal. Calcd for $C_{15}H_{20}N_2O_9 \cdot 0.5H_2O$: C, 48.85; H, 5.64; N, 7.13. Found: C, 48.36; H, 5.67; N, 7.58.

1-(Tri-O-acetyl-β-D-ribofuranosyl)thymine (XXXIII), To a solution of 135 mg of 1-(β-D-ribofuranosyl)thymine[31] (0.58 mmole) in 4 ml of pyridine was added 0.2 ml of acetic anhydride. After standing 16 hr at room temperature, the solvent was evaporated *in vacuo* and the resulting colorless syrup dissolved in chloroform. The chloroform solution was washed twice with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give a syrupy XXXIII (164 mg, 74%). Purification by chromatography on a silica gel column (95 × 22 mm) using 5% methanol–chloroform as eluent yielded a syrup which showed one spot on tlc R_f 0.81, system A, and R_f 0.78, system B. Nmr spectrum in τ (DMSO-d_6): singlet, 2.37 (H_6); doublet, 4.02 ($H_{1'}$, $J_{1',2'}$ = 4.3 cps); multiplet, 4.51 ($H_{2'}$, $H_{3'}$); broad peak, 5.63 ($H_{4'}$, $H_{5'}$, $H_{5'}$); singlet, 8.18 (C_5-methyl); acetyl peaks 7.90 and 7.92 (2) (in $CDCl_3$ 7.84, 7.86, and 7.90).

Preparation of 5,6-Dihydro Derivatives. General Procedure, The 5,6-dihydro derivatives were prepared by the method of Cohn and Doherty.[32] Samples of 43–300 mg were dissolved in 50 ml of ethanol and treated with one-half their weight with 5% rhodium on alumina catalyst. The samples were hydrogenated at room temperature and atmospheric pressure until the theoretical amount of hydrogen had been absorbed. The products were examined by ultaviolet spectroscopy, and the absence of selective absorption at 260 mμ showed that the hydrogenation of the 5,6 double bond was essentially complete. The results are summarized in Table IV.

1-(β-D-Ribofuransoyl)-5,6-dihydrouracil, A 1-g (4.1 mmoles) sample of uridine was dissolved in 50 ml of water. The sample

(28) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(29) R. Fecher, J. F. Codington, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 1889 (1961).

(30) N. C. Yung and J. J. Fox, *ibid.*, **83**, 3060 (1961).

(31) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *ibid.*, **78**, 2117 (1956).

(32) W. E. Cohn and D. G. Doherty, *ibid.*, 78, 2863 (1956).

Table IV, Physical and Microanalytical Data For 5,6-Dihydro Nucleosides

5,6-Dihydro derivative	Starting material; 1-β-D-derivatives of	Solvent(s) of crystallization	% yield	Mp, °C, cor	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
IV	3′-Acetamido-2′-O-acetyl-3′-deoxyglucopyranosyluracil (III)	Ethanol	75	265–267	$C_{14}H_{21}N_3O_8$	46.79	5.89	11.69	46.32	5.44	11.40
VIII	3′-Acetamidotri-O-acetyl-3′-deoxymannopyranosyluracil (VII)	Acetone	61	226–228.5	$C_{18}H_{25}N_3O_{10}$	48.75	5.68	9.48	48.67	5.60	10.33
X	3′-Acetamido-2′-O-acetyl-3′-deoxymannopyranosyluracil (IX)	Ethanol		232–235 dec	$C_{14}H_{21}N_3O_8$	46.79	5.89	11.69	46.27	5.46	11.87
XII	3′-Acetamidotri-O-acetyl-3′-deoxygalactopyranosyluracil (XI)	Ethanol–ether	100	210–213 dec	$C_{18}H_{25}N_3O_{10}$	48.75	5.68	9.48	48.95	5.43	8.97
XVIII	3′-Acetamido-3′-deoxymannopyranosyluracil (XVII)	Methanol–ethanol–water	38	214.5–216	$C_{12}H_{19}N_3O_7 \cdot 0.5H_2O$[f]	44.18	6.15	12.87	44.16	6.01	12.64
XXII	3′,5′-Di-O-acetyl-2′-deoxyuridine (XXI)[a]	Syrup	74		$C_{13}H_{18}N_2O_7$	49.68	5.77	8.92	49.05	6.24	8.36
XXIV	2′,3′,5′-Tri-O-acetyluridine (XXIII)[b]	Syrup[c]			$C_{15}H_{20}N_2O_9$	48.40	5.42	7.53	48.88	5.67	7.36
XXVI	Tri-O-acetylarabinofuranosyluracil (XXV)	Syrup[d]	33		$C_{15}H_{20}N_2O_9 \cdot 0.7CH_3CH_2OH$[f]	47.44	6.16	7.05	47.56	5.93	6.73
XXVIII	Tri-O-acetyllyxofuranosyluracil (XVII)	Syrup[e]	37		$C_{15}H_{20}N_2O_9$	48.40	5.42	7.53	48.80	5.67	7.99
XXX	Tri-O-acetylxylofuranosyluracil (XXIX)	Syrup[g]	98		$C_{15}H_{20}N_2O_9$	48.40	5.42	7.53	48.55	5.84	7.46
XXXII	Tri-O-acetylxylofuranosylthymine (XXXI)	Mixture									
XXXIV	Tri-O-acetylribofuranosylthymine (XXXIII)	Syrup[h]	97		$C_{16}H_{22}N_2O_9$	49.73	5.74	7.25			

[a] R. J. Cushley, J. F. Codington, and J. J. Fox, manuscript in preparation. [b] D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956). We are indebted to Miss I. Doerr for a sample of this compound. [c] The product was purified on a silica gel column, using methanol–chloroform (1:9). Tlc R_f 0.30, solvent system A. [d] The product was purified on a silica gel column, using gradient elution 0–10% methanol in chloroform. Tlc R_f 0.49, solvent system A. [e] The colorless product was dissolved in chloroform, washed with water, and dried over Na_2SO_4. Tlc R_f 0.58, solvent system A. [f] Solvent of crystallization confirmed by nmr. [g] The product was purified on a silica gel column using gradient elution 0–5% methanol in chloroform. Tlc R_f 0.76, solvent system A; R_f 0.77, solvent system B. [h] The product was purified on a silica gel column using gradient elution 0–6% methanol in chloroform. Tlc R_f 0.74, solvent system A; R_f 0.55, solvent system B.

was hydrogenated over 360 mg of 5% rhodium on alumina[32] catalyst at 24.5° and atmospheric pressure. There ensued a rapid hydrogen uptake of 100 ml (theory 100 ml). The product, a white solid which was very hygroscopic, contained no selective absorption at 260 mμ in the ultraviolet, $[\alpha]_D$ −33° (*c*, 2.2 in water) (lit.[33] $[\alpha]_D$ +39.1°). Nmr spectrum in τ (D_2O, external TMS): doublet, 4.10 ($H_{1'}$, $J_{1'2'}$ = 6 cps); multiplet, 5.80 ($H_{2'}$, $H_{3'}$, $H_{4'}$); multiplet, 6.21 ($H_{5'}$,$H_{5''}$); triplet, 6.40 (H_6,H_6); triplet, 7.22 (H_5,H_5, splitting 6.6 cps); and no peaks in the olefin region.

(33) P. A. Levene and F. B. La Forge, *Chem. Ber.*, **45**, 608 (1912).

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